

WHAT IS CLAIMED:

- Sub
A-1
1. A transgenic ungulate bearing a homozygous deletion or disruption of the prion gene, wherein said deletion or disruption prevents expression of a functional endogenous prion protein, and wherein lack of expression of a functional endogenous prion protein renders said bovine unsusceptible to prion-related diseases.
2. The transgenic ungulate of Claim 1, wherein said deletion or disruption is created by homologous recombination of heterologous DNA into the prion gene locus.
3. The transgenic ungulate of Claim 2, wherein said heterologous DNA comprises a selectable marker.
4. The transgenic ungulate of Claim 3, wherein said heterologous DNA comprises a neomycin resistance gene operably linked to a PGK promoter.
5. The transgenic ungulate of Claim 3, wherein said heterologous DNA comprises a promoterless neomycin resistance gene.
6. The transgenic ungulate of Claim 1, wherein said ungulate is a bovine.
7. The transgenic ungulate of Claim 6, wherein said prion-related disease is bovine spongiform encephalitis (BSE).
- 25
Sub A-1
8. The transgenic bovine of Claim 7, wherein said bovine bears a heterologous gene that is extraneous to the prion gene locus.
9. The transgenic bovine of Claim 8, wherein the heterologous gene is operably linked to a mammary-specific promoter, and expression of said heterologous gene enables production of a recombinant protein in the milk of said transgenic bovine.

10. An isolated DNA molecule comprising at least part of an ungulate prion gene promoter operably linked to either a selectable marker gene coding region or a reporter gene coding region.

11. The isolated DNA molecule of Claim 10, wherein said molecule further comprises a second ungulate DNA sequence from or adjacent to the ungulate prion gene locus, wherein said prion promoter region and said second DNA sequence facilitate homologous recombination of said selectable marker into the ungulate genome such that said prion gene is disrupted or deleted.

12. The isolated DNA molecule of Claim 10, wherein said selectable marker is a neomycin resistance gene.

13. The isolated DNA molecule of Claim 12 further comprising a thymidine kinase gene.

14. A plasmid vector comprising the isolated DNA molecule of Claim 10.

15. A DNA targeting molecule capable of specifically and functionally deleting or disrupting expression of an ungulate prion gene, wherein said disruption occurs by homologous recombination into said ungulate prion gene locus.

16. The DNA targeting molecule of Claim 15, wherein the targeting molecule facilitates the deletion or disruption of exon 3 of the prion gene.

17. The DNA targeting molecule of Claim 15, wherein said targeting molecule comprises a selectable marker gene.

18. The DNA targeting molecule of Claim 17, wherein said selectable marker gene is a neomycin resistance gene.

19. The DNA targeting molecule of Claim 18, further comprising a thymidine kinase gene.

5 20. A plasmid vector comprising the DNA targeting molecule of Claim 19.

21. The plasmid vector of Claim 20, wherein said ungulate prion gene is the bovine prion gene.

10 22. A cloned transgenic ungulate having the same genotype as the transgenic ungulate of Claim 1, wherein said cloned transgenic ungulate is created using nuclear transfer techniques.

15 23. The cloned transgenic ungulate of Claim 22, wherein said ungulate bears a heterologous gene that is extraneous to the prion gene locus.

24. The transgenic ungulate of Claim 23, wherein said ungulate is a bovine.

20 25. The transgenic ungulate of Claim 24, wherein the heterologous gene is operably linked to a mammary-specific promoter, and expression of said heterologous gene enables production of a therapeutic protein in the milk of said transgenic bovine.

25 26. A line of transgenic ungulates having the same genotype as the transgenic ungulate of Claim 1.

27. A line of transgenic ungulates having the same genotype as the cloned transgenic ungulate of Claim 22.

30 28. A transgenic ungulate comprising a targeted gene deletion.

29. A transgenic bovine comprising a targeted gene deletion.

00016546-032601

30. A transgenic ungulate bearing a heterozygous deletion of the prion gene, wherein said deletion prevents expression of a functional endogenous prion protein from one prion gene allele, and wherein lack of expression of a functional endogenous prion protein from said one allele renders said ungulate less susceptible to prion-related diseases.

31. A method of making the transgenic ungulate of Claim 30, comprising the steps of:

- (1) isolating genomic DNA from ungulate cells;
- (2) isolating a prion gene allele from said genomic DNA;
- (3) determining a restriction enzyme map and the intron/ exon structure of the ungulate prion gene allele isolated from said ungulate genomic DNA;
- (4) sub-cloning fragments from said prion gene allele for the construction of a targeting DNA molecule;
- (5) constructing a targeting DNA molecule which is capable of disrupting or deleting an ungulate prion gene allele by homologous recombination;
- (6) transfecting said ungulate cells such that homologous recombinants are isolated;
- (7) transferring the nuclei from a transfected cell containing the targeting molecule homologously recombined into a prion gene allele to the cytoplasm of an enucleated mature ungulate oocyte;
- (8) culturing said oocyte to form a blastocyst; and
- (9) transferring said blastocyst to a recipient ungulate such that a transgenic ungulate according to Claim 30 is born.

32. A method of making the transgenic ungulate of Claim 1, comprising the steps of:

- (1) isolating genomic DNA from ungulate cells;
- (2) isolating a prion gene allele from said genomic DNA;

- (3) determining a restriction enzyme map and the intron/ exon structure of the bovine prion gene allele isolated from said ungulate genomic DNA;
 - (4) sub-cloning fragments from said prion gene allele for the construction of a targeting DNA molecule;
 - 5 (5) constructing a targeting DNA molecule which is capable of disrupting or deleting an ungulate prion gene allele by homologous recombination;
 - (6) transfecting said ungulate cells such that homologous recombinants are isolated;
 - (7) transferring the nuclei from a transfected cell containing the targeting
10 molecule homologously recombined into a prion gene allele to the cytoplasm of an enucleated mature ungulate oocyte;
 - (8) culturing said oocyte to form a blastocyst;
 - (9) transferring said blastocyst to a recipient ungulate such that a transgenic ungulate having a heterozygous deletion or disruption in the prion gene is born; and
 - 15 (10) breeding said transgenic ungulate having a heterozygous deletion or disruption in the prion gene to obtain the transgenic ungulate of Claim 1, or targeting a deletion of the other allele using primary fibroblasts derived from a heterozygous transgenic ungulate to create the transgenic ungulate of Claim 1.
-
- 20 33. The method of Claim 31, wherein said ungulate cells are bovine cells.
 34. The method of Claim 32, wherein said ungulate cells are bovine cells.
 35. The method of Claim 33, wherein said bovine cells are derived from
25 fetal fibroblast cells.
 36. The method of Claim 34, wherein said bovine cells are derived from fetal fibroblast cells.
 - 30 37. The method of Claim 35, wherein said cells are BFF cells.

~~38.~~ The method of Claim 36, wherein said cells are BFF cells.

~~39.~~ The transgenic ungulate of Claim 1, wherein said ungulate bears a heterologous gene that is extraneous to the prion gene locus.

40. The transgenic ungulate of Claim 39, wherein said extraneous heterologous gene is a mutant prion gene allele which confers an increased tendency to develop a prion-related spongiform encephalopathy.

41. The cloned transgenic ungulate of Claim 23, wherein said extraneous heterologous gene is a mutant prion gene allele which confers an increased tendency to develop a prion-related spongiform encephalopathy.

42. A method of using the transgenic ungulate of Claim 40 to screen for or evaluate agents which may be used in the treatment or prevention of spongiform encephalopathies, said method comprising:

- (1) administering a putative therapeutic agent to said transgenic ungulate before or after the development of said prion-related spongiform encephalopathy; and
- (2) monitoring said ungulate to determine whether said prion-related spongiform encephalopathy has been prevented or treated.

43. A method of xenotransplantation using fetal tissue or cells derived from the transgenic ungulate of Claim 1, said method comprising:

- (1) generating a transgenic fetus having the same genotype as the transgenic ungulate of claim 1, either by mating or cloning techniques;
- (2) isolating tissue or cells of interest from said fetus; and
- (3) transplanting the fetal tissue or cells into a recipient mammal.

44. The method of Claim 43, wherein said cells are fetal neurons.

00016516:022601
FO9220:9159T800

45. The method of Claim 44, wherein said cells are derived from fetal corneal tissue.

46. The transgenic ungulate of Claim 1, wherein said ungulate bears at least one other deletion or disruption that is extraneous to the prion gene locus.

47. The transgenic bovine of Claim 46, wherein said at least one other deletion or disruption is in a gene which interferes with xenotransplantation.

48. A method of xenotransplantation using fetal tissue or cells derived from the transgenic bovine of Claim 44, said method comprising:

- (1) generating a transgenic fetus having the same genotype as the transgenic bovine of claim 1, either by mating or cloning techniques;
- (2) isolating tissue or cells of interest from said fetus; and
- (3) transplanting the fetal tissue or cells into a recipient mammal.

49. A method of using the transgenic bovine of Claim 8 for the production of recombinant proteins, said method comprising:

- (1) generating a female transgenic bovine according to claim 8; and
- (2) isolating the recombinant protein from the milk of said transgenic bovine.